In the Claims:

Claim 4, line 3 insert a comma -- , -- after "promoter".

Claim 7, line 4 insert a comma -- , -- after "promoter".

Claim 10, line 7 insert a comma -- , -- after "promoter".

Claim 13, line 6 insert a comma -- , -- after "promoter".

Remarks

Rejection under 35 U.S.C. §112

Claims 4, 7, 10 and 13 were rejected as being indefinite under 35 USC §112. These claims as amended have a comma inserted before the phrase "and a structural sequence" as suggested by the Examiner. Accordingly, this rejection is believed to be obviated and withdrawal of this rejection is solicited.

Rejection under 35 U.S.C. §103

Claims 1-15 stand rejected under 35 USC §103 as being unpatentable over Anderson taken with Guilley et al. It is the position of the Patent Office that the Anderson patent teaches a method for transforming plant cells with a chimeric gene comprising a viral promoter from the thymidine kinase gene (from Herpes Simplex Virus) and a bacterially-derived kanamycin resistance structural gene sequence heterologous to said promoter, wherein the transformation was carried out using a plasmid comprising T-DNA borders from an *Agrobacterium tumefaciens* plasmid, and wherein transformed kanamycin-resistant plant cells

expressing the chimeric gene were recovered. The Patent Office correctly recognizes that Anderson does not teach nor suggest heterologous gene expression driven by the CaMV35S or CaMV19S promoters. Guilley et al. is asserted to teach the identification of the CaMV35S and CaMV19S promoters of the CaMV and their relative strengths. Based on these two references, the Patent Office maintains that it would have been obvious to one skilled in the art to utilize the viral promoter mediated method for plant transformation taught by Anderson, and to modify that method for plant transformation by incorporating the CaMV35S or CaMV19S promoters allegedly taught by Guilley et al. The position of the Patent Office is respectfully deemed to be ill-founded and is traversed in view of the following remarks and corroborating declaration by Dr. Charles Armstrong submitted herewith.

Numerous reasons to doubt the teaching of the Anderson patent for which it is relied upon by the Office in the above-referenced rejection of the present invention exist now and at the time of the Anderson filing. The Anderson patent offers as its demonstration that the Thymidine Kinase (TK) viral promoter drove the expression of a structural gene encoding for neomycin phosphotransferase by a showing that "up to 5%" of the treated (tobacco) protoplasts cleaved and produced cell masses with as many as 16 cells" in the presence of 50 μg/ml kanamycin sulfate. No verifying data such as enzyme studies, Western blots or the like were provided to further support the alleged results.

It has been shown in Applicants' laboratories that a percentage of cells can escape kanamycin selection and go on to be regenerated to whole plants even though the ells have not been transformed with the kanamycin resistance gene. This is particularly true at the low level of kanamycin (50 μg/ml) utilized by Anderson (see Horsch declaration discussed infra).

A position has been taken by the Patent Office that due to the differences which exist in the genetic construct of the Horsch declaration (previously submitted to the Office on December 7, 1990) and that taught by Anderson, the applicability of the results is made unclear. Quite to the contrary, the Applicants have where possible attempted to make a comparison in a manner which would give a thymidine kinase construct suggested by Anderson the benefit of the doubt when assaying the utility of such a construct in plant transformation/ regeneration.

- The Examiner has noted that the Horsch construct did contain a polyoma virus enhancer sequence. This element is known to be operably inserted into the construct (as purchase from Stratagene), and therefore as stated by Dr. Horsch in the above referenced declaration, if the promoter did indeed function in plant cells then one would only expect the enhancer sequence to further elevate such performance. The Office has provided no scientific basis on which to challenge this statement.

- The Horsch construct contains a 3' nontranslated polyadenylation signal. The Anderson construct did not contain such a signal. The Examiner will certainly recognize that the absence of the 3' signal would severely impair gene function by lowering the ability of the mRNA to be transported to the cytoplasm to be subsequently transcribed into protein.

Enclosed herewith is a declaration of Dr. Charles Armstrong. Dr. Armstrong has reviewed the experiments discussed in the previously submitted Horsch declaration and also concludes that these results demonstrate that the thymidine kinase driven kanamycin resistance gene is for all practical purposes "inoperative" in plant cells. That is, growth of plant tissue in the presence of kanamycin is almost completely inhibited in the presence or absence of the thymidine kinase driven chimeric gene suggested by Anderson. It is difficult, if not impossible, to understand how a credible difference can be attributed to the presence of the thymidine kinase promoter driven NPTII gene.

Applicants submit that the conclusion reached by Dr. Horsch and now corroborated by Dr. Armstrong is correct. Concurrence with this conclusion does not require the Office to accept a position that in any way is contrary to scientific evidence as a whole. Rather, the use of non-plant promoters as the regulatory region of a chimeric gene for expression in plants has also been shown by other researchers to be incapable of driving expression of a gene in plant cells. This fact is made clear and supported in the Rogers' declaration (filed with Applicants'

December 19, 1989 response to the July 27, 1989 Office Letter in the parent application U.S.S.N. 06/931,492) and accompanying citations, especially the Caplan reference. The Caplan reference states on page 818, second column, first full paragraph:

> Tn7(40) and Tn5(11) were inserted in vivo into the T-DNA of Ti plasmids pTiT37 and pTiA6NC, respectively, and were found to be efficiently cotranscribed with the T-DNA. The genes encompasses by these transposons failed to be expressed, presumably because the eukaryotic transcriptional machinery of the plant did not recognize the promoter sequences of these prokaryotic genes. Further attempts to express heterologous eukaryotic genes, such as the yeast alcohol dehydrogenase gene (23) or genes from mammalian cells, such as ß-globin (44), interferon (45) and genes under control of the SV40 early promoter (46), showed that none of these genes was transcribed in plant cells. This suggests that specific transcription factors or signals that are required for their expression are present only in the cells or specific tissues of their original hosts. (emphasis added)

As Caplan et al. states, "genes under the control of the SV40 early promoter (46), showed that none of these genes was transcribed in plant cells." The SV40

early promoter is a viral promoter from the Papovavirus group of animal viruses. The citation accompanying this statement (46) mentions that the article providing data to support this research is "in preparation." A search for an article by the authors named in the citation turned up no such published article, but the conclusion is drawn that other researchers in the field were unable to utilize non-plant promoters to drive gene expression in plant cells. Thus, one of ordinary skill in the art was confronted with conflicting data as to whether or what types of viral promoters would or would not work in plant systems. Researchers experimenting with viral promoters therefore had no reasonable expectations of success as to what would or would not work in plant systems.

The Examiner seems to be of the opinion that to find the teaching of Anderson patent inadequate to combine with Guilley et al. to render the present invention unpatentable under §103 requires that the Examiner hold the Anderson patent invalid. The undersigned attorney cannot see where such a determination is necessary. The Examiner need only conclude that the teaching of Anderson and Guilley et al. in view of the evidence of record do not render the claimed invention unpatentable.

The Patent Office's reliance upon Guilley et al. for its alleged teaching of the relative promoter strength for the CaMV promoter is misplaced. It is impossible to conclude anything about viral promoter strength from infected plant cells since those cells contain many, many copies of the subject promoter.

Because there are so many copies of the subject gene (and promoter) it was equally possible given the Guilley et al. data that expression from each of these gene copies was not high but that weak expression from each copy was amplified by the presence of the other gene copies. That is, it could have been reasonably concluded that the CaMV19S and 35S promoters were weak promoters and seemingly provide high levels of expression (i.e., large amounts of RNA) because the genes are amplified (i.e., many copies).

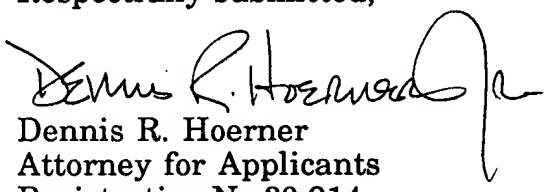
Guilley et al.'s use of the 35S and 19S promoter is also distinguishable from Applicants' use in that Guilley et al. describes in vitro transcription in HeLa cells. The HeLa cell transcription system is a mammalian cell lysate system which is not a plant cell environment. The fact that a large CaMV fragment can effect transcription in a HeLa cell lysate system provides no likelihood or expectation of success that a small CaMV fragment isolating the 35S or 19S promoter from CaMV would be effective as a promoter in a chimeric gene when incorporated into a plant cell's genome. Moreover, while this study permitted the identification of a 35S transcript, the 19S transcript which is easily detectable in infected plants, could not be mapped "presumably due to the low level of *in-vitro* transcription from this promoter." Since this result of Guilley et al. shows that the *in-vitro* system does not mimic the *in planta* result for the 19S promoter, it should not be given the scientific credibility to be a valid indicator assay for the performance of the CaMV35S or CaMV19S promoters in plants.

In summary, the subject matter of Claims 1-15 may have been "obvious to try" but certainly are not obvious in view of Anderson and Guilley et al. and the §103 rejection should therefore be withdrawn. Such action is respectfully requested.

Claims 16-18 have been rejected under §103 as being unpatentable over Anderson taken with Guilley et al. as applied previously and further in view of Zambryski et al. This rejection is respectfully traversed. The inadequacies of Anderson and Guilley et al. have been discussed above and will not be repeated here. Zambryski et al. is asserted as teaching the regeneration of whole plants from cells transformed with a vector containing chimeric genes, T-DNA borders and deleted tumor genes. Nothing in Zambryski suggests a differentiated plant capable of expressing a polypeptide through performance of a differentiated plant capable of expressing a polypeptide through performance of a sequence of cellular steps including transcription of a chimeric gene that includes a promoter selected from the CaMV35S or CaMV19S promoter. In light of the discussion above distinguishing Applicants' invention from the teachings of Anderson and Guilley, Zambryski does not add the sufficient teachings to render Applicants' claimed invention obvious. Therefore it is believed that the §103 rejection as it pertains to claims 16-18 should also be withdrawn. Such action is respectfully solicited.

In view of the foregoing amendment, remarks, and Armstrong declaration it is believed that this application is now in condition for formal allowance. Such action in the regular course of business is respectfully requested. If the Examiner believes a phone conference would be beneficial to the quick allowance of this case, he is requested to phone Applicants' attorney at the number listed below.

Respectfully submitted,



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